Figure 1: Alignment of INSP105 against placental growth hormone (*Homo sapiens*).

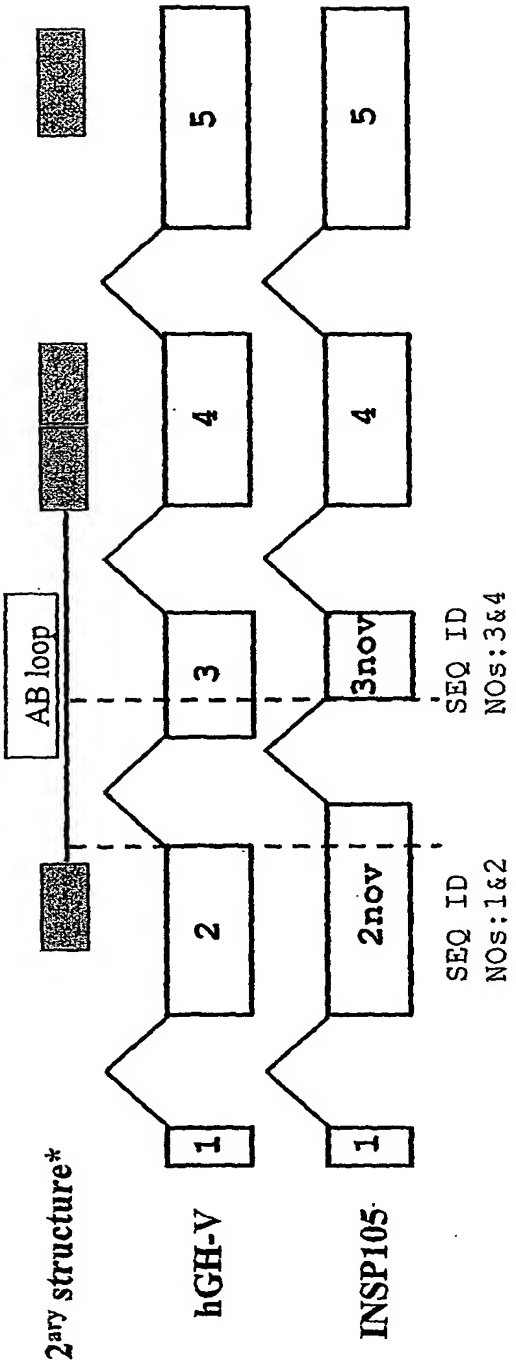
```
Query: 1    MAAGSRTSLLLAFGLLCLSWLQEGSAFPTIPLSRLFDNAMLRARRLYQLAYDTYQEFVSS 60
            MAAGSRTSLLLAFGLLCLSWLQEGSAFPTIPLSRLFDNAMLRARRLYQLAYDTYQEF  +
Sbjct: 1    MAAGSRTSLLLAFGLLCLSWLQEGSAFPTIPLSRLFDNAMLRARRLYQLAYDTYQEFEEA 60
                                                                    ***

Query: 61   WVM------------------ESIPTPSNRVKTQQKSNLELLRISLLLIQSWLEPVQLLR 102
            +++                  ESIPTPSNRVKTQQKSNLELLRISLLLIQSWLEPVQLLR
Sbjct: 61   YILKEQKYSFLQNPQTSLCFSESIPTPSNRVKTQQKSNLELLRISLLLIQSWLEPVQLLR 120
            ************************************

Query: 103  SVFANSLVYGASDSNVYRHLKDLEEGIQTLMWRLEDGSPRTGQIFNQSYSKFDTKSHNDD 162
            SVFANSLVYGASDSNVYRHLKDLEEGIQTLMWRLEDGSPRTGQIFNQSYSKFDTKSHNDD
Sbjct: 121  SVFANSLVYGASDSNVYRHLKDLEEGIQTLMWRLEDGSPRTGQIFNQSYSKFDTKSHNDD 180

Query: 163  ALLKNYGLLYCFRKDMDKVETFLRIVQCRSVEGSCGF 199
            ALLKNYGLLYCFRKDMDKVETFLRIVQCRSVEGSCGF
Sbjct: 181  ALLKNYGLLYCFRKDMDKVETFLRIVQCRSVEGSCGF 217
```

Figure 2: Gene Structure



*Secondary structure is based on hGH-N structure

Figure 3: Predicted nucleotide sequence of INSP105 with translation

```
   1  atggctgcag gctcccggac gtccctgctc ctggcttttg gcctgctctg
        m  a  a  g  s  r  t  s  l  l  l  a  f  g  l  l

  51  cctgtcctgg cttcaagagg gcagtgcctt cccaaccatt cccttatcca
      c  l  s  w  l  q  e  g  s  a  f  p  t  i  p  l  s

 101  ggctttttga caacgctatg ctccgcgccc gtcgcctgta ccagctggca
       r  l  f  d  n  a  m  l  r  a  r  r  l  y  q  l  a

 151  tatgacacct atcaggagtt tgtaagctct tgggtaatgg agtctattcc
       y  d  t  y  q  e  f  v  s  s  w  v  m  e  s  i

 201  aacaccttcc aacagggtga aaacgcagca gaaatctaac ctagagctgc
      p  t  p  s  n  r  v  k  t  q  q  k  s  n  l  e  l

 251  tccgcatctc cctgctgctc atccagtcat ggctggagcc cgtgcagctc
       l  r  i  s  l  l  l  i  q  s  w  l  e  p  v  q  l

 301  ctcaggagcg tcttcgccaa cagcctggtg tatggcgcct cggacagcaa
       l  r  s  v  f  a  n  s  l  v  y  g  a  s  d  s

 351  cgtctatcgc cacctgaagg acctagagga aggcatccaa acgctgatgt
      n  v  y  r  h  l  k  d  l  e  e  g  i  q  t  l  m

 401  ggaggctgga agatggcagc ccccggactg ggcagatctt caatcagtcc
       w  r  l  e  d  g  s  p  r  t  g  q  i  f  n  q  s

 451  tacagcaagt ttgacacaaa atcgcacaac gatgacgcac tgctcaagaa
       y  s  k  f  d  t  k  s  h  n  d  d  a  l  l  k

 501  ctacgggctg ctctactgct tcaggaagga catggacaag gtcgagacat
      n  y  g  l  l  y  c  f  r  k  d  m  d  k  v  e  t

 551  tcctgcgcat cgtgcagtgc cgctctgtgg agggcagctg tggcttc
       f  l  r  i  v  q  c  r  s  v  e  g  s  c  g  f
```

Bold = signal sequence

Figure 4: INSP105 coding exon organization in genomic DNA and position of PCR primers

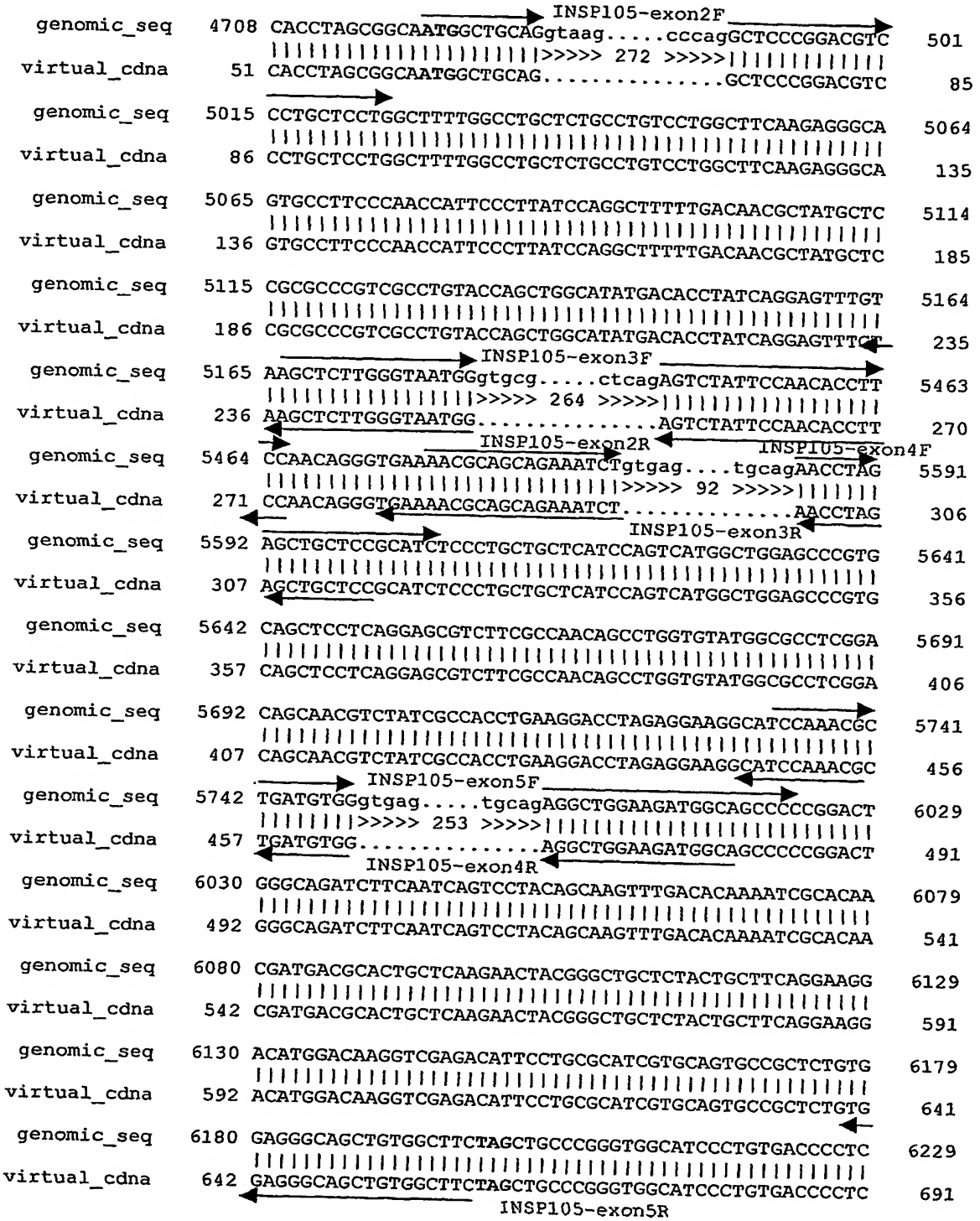


Figure 5: Alignment of INSP105 with pENTR clone–miniprep 6 indicating the position of PCR primers used to re-amplify the correct 5' end of the cDNA.

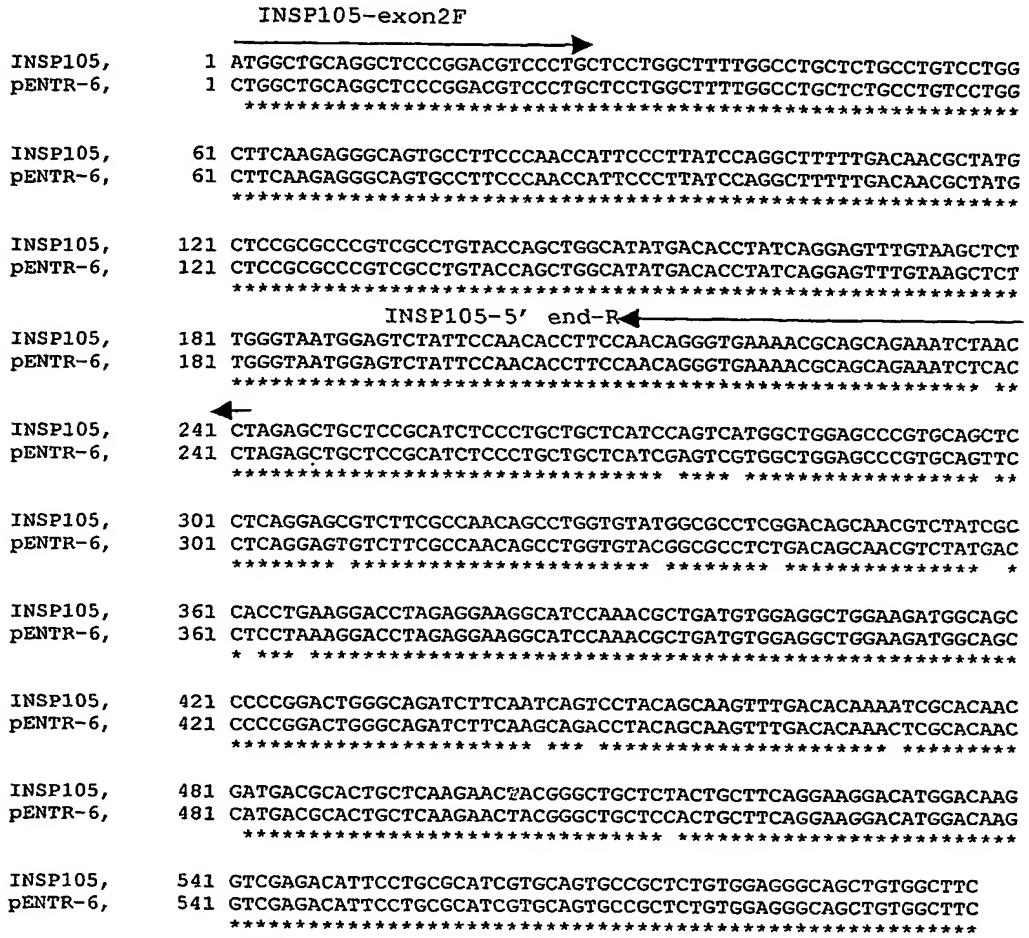


Figure 6: Alignment of INSP105 with pENTR clone–miniprep 10 indicating the position of PCR primers used to re-amplify the correct central region of the cDNA.

```
INSP105       1 ATGGCTGCAGGCTCCCGGACGTCCCTGCTCCTGGCTTTTGGCCTGCTCTGCCTGTCCTGG
pENTR-10      1 ATGGCT---------CCGGACGTCCCTGCTCCTGGCTTTTGGCCTGCTCTGCCTGCCCTGG
                ******          ********************************** *****

INSP105      61 CTTCAAGAGGGCAGTGCCTTCCCAACCATTCCCTTATCCAGGCTTTTTGACAACGCTATG
pENTR-10     61 CTTCAAGAGGGCAGTGCCTTCCCAACCATTCCCTTATCCAGGCTTTTTGACAACGCTATG
                ************************************************************

INSP105     121 CTCCGCGCCCGTCGCCTGTACCAGCTGGCATATGACACCTATCAGGAGTTTGTAAGCTCT
pENTR-10    121 CTCCGCGCCCATCGTCTGCACCAGCTGGCCTTTGACACCTACCAGGAGTTTGTAAGCTCT
                ********** *** *** ********** * ********* ******************
                                  INSP105-center-F ──────────────────────►
INSP105     181 TGGGTAATGGAGTCTATTCCAACACCTTCCAACAGGGTGAAAACGCAGCAGAAATCTAAC
pENTR-10    181 TGGGTAATGGAGTCTATTCCAACACCTTCCAACAGGGTGAAACCGCAGCAGAAATCTAAC
                ****************************************** *****************
                ►
INSP105     241 CTAGAGCTGCTCCGCATCTCCCTGCTGCTCATCCAGTCATGGCTGGAGCCCGTGCAGCTC
pENTR-10    241 CTAGAGCTGCTCCGCATCTCCCTGCTGCTCATCCAGTCATGGCTGGAGCCCGTGCAGCTC
                ************************************************************

INSP105     301 CTCAGGAGCGTCTTCGCCAACAGCCTGGTGTATGGCGCCTCGGACAGCAACGTCTATCGC
pENTR-10    301 CTCAGGAGCGTCTTCGCCAACAGCCTGGTGTATGGCGCCTCGGACAGCAACGTCTATCGC
                ************************************************************
                                  INSP105-center-R ◄──────────────────────
INSP105     361 CACCTGAAGGACCTAGAGGAAGGCATCCAAACGCTGATGTGGAGGCTGGAAGATGGCAGC
pENTR-10    361 CACCTGAAGGACCTAGAGGAAGGCATCCAAACGCTGATGTGGAGGCTGGAAGATGGCAGC
                ************************************************************
                ◄
INSP105     421 CCCCGGACTGGGCAGATCTTCAATCAGTCCTACAGCAAGTTTGACACAAAATCGCACAAC
pENTR-10    421 CACCTGACTGGGCAGACCCTCAAGCAGACCTACAGCAAGTTTGACACAAACTCGCACAAC
                * ** *********** * **** *** ********************** *********

INSP105     481 GATGACGCACTGCTCAAGAACTACGGGCTGCTCTACTGCTTCAGGAAGGACATGGACAAG
pENTR-10    481 CATGACGCACTGCTCAAGAACTACGGGCTGCTCCACTGCTTCAGGAAGGACATGGACAAG
                 ******************************** ************************

INSP105     541 GTCGAGACATTCCTGCGCATCGTGCAGTGCCGCTCTGTGGAGGGCAGCTGTGGCTTC
pENTR-10    541 GTCGAGACATTCCTGCGCATCGTGCAGTGCCGCTCTGTGGAGGGCAGCTGTGGCTTC
                ************************************************************
```

Figure 7: Alignment of INSP105 with pENTR clone–miniprep 3 indicating the position of PCR primers used to re-amplify the correct 3' end of the cDNA.

```
INSP105      1 ATGGCTGCAGGCTCCCGGACGTCCCTGCTCCTGGCTTTTGGCCTGCTCTGCCTGTCCTGG
pENTR-3      1 ATGGCTGNAGGNTCCCGGACGTCCCTGNTCCTGGCTTTTGGCCTGGTCTGNCTGNCCTGG
               ******* *** *************** ***************** **** *** *****

INSP105     61 CTTCAAGAGGGCAGTGCCTTCCCAACCATTCCCTTATCCAGGCTTTTTGACAACGCTATG
pENTR-3     61 CTTCAAGAGGGCAGTGNCTTCCCAACCATTCCCTTATCCAGGCTTTTTGACAACGCTATG
               ***************** ****************************************

INSP105    121 CTCCGCGCCCGTCGCCTGTACCAGCTGGCATATGACACCTATCAGGAGTTTGTAAGCTCT
pENTR-3    121 CTCCGCGCCCGTCGCCTGTACCAGCTGGCATATGACACCTATCAGGAGTTTGTAAGCTCT
               ************************************************************

INSP105    181 TGGGTAATGGAGTCTATTCCAACACCTTCCAACAGGGTGAAAACGCAGCAGAAATCTAAC
pENTR-3    181 TGGGTAATGGAGTCTATTCCAACACCTTCCAACAGGGTGAAAACGCAGCAGAAATCTAAC
               ************************************************************

INSP105    241 CTAGAGCTGCTCCGCATCTCCCTGCTGCTCATCCAGTCATGGCTGGAGCCCGTGCAGCTC
pENTR-3    241 CTAGAGCTGCTCCGCATC----------------------------------------
               ******************

INSP105    301 CTCAGGAGCGTCTTCGCCAACAGCCTGGTGTATGGCGCCTCGGACAGCAACGTCTATCGC
pENTR-3    301 ------------------------------------------------------------

                        INSP105-3' end-F ──────────►
INSP105    361 CACCTGAAGGACCTAGAGGAAGGCATCCAAACGCTGATGTGGAGGCTGGAAGATGGCAGC
pENTR-3    361 ----------------------------CAAACGCTGATGTGGAGGCTGGAAGATGGCAGC
                                            *********************************

INSP105    421 CCCCGGACTGGGCAGATCTTCAATCAGTCCTACAGCAAGTTTGACACAAAATCGCACAAC
pENTR-3    421 CCCCGGACTGGGCAGATCTTCAATCAGTCCTACAGCAAGTTTGACACAAAATCGCACAAC
               ************************************************************

INSP105    481 GATGACGCACTGCTCAAGAACTACGGGCTGCTCTACTGCTTCAGGAAGGACATGGACAAG
pENTR-3    481 GATGACGCACTGCTCAAGAACTACGGGCTGCTCTACTGCTTCAGGAAGGACATGGACAAG
               ************************************************************

INSP105    541 GTCGAGACATTCCTGCGCATCGTGCAGTGCCGCTCTGTGGAGGGCAGCTGTGGCTTC
pENTR-3    541 GTCGAGACATTCCTGCGCATCGTGCAGTGCCGCTCTGTGGAGGGCAGCTGTGGCTTC
               ************************************************************
                                    ◄──────────
                          INSP105-3' end-R
```

Figure 8: Nucleotide sequence and translation of cloned INSP105 ORF.

```
   1  acaagtttgt acaaaaaagc aggcttcgcc accatggctg caggctcccg
                                          m  a  a  g  s

  51  gacgtccctg ctcctggctt ttggcctgct ctgcctgtcc tggcttcaag
      r  t  s  l  l  l  a  f  g  l  l  c  l  s  w  l  q

 101  agggcagtgc cttcccaacc attcccttat ccaggctttt tgacaacgct
       e  g  s  a  f  p  t  i  p  l  s  r  l  f  d  n  a

 151  atgctccgcg cccgtcgcct gtaccagctg gcatatgaca cctatcagga
        m  l  r  a  r  r  l  y  q  l  a  y  d  t  y  q

 201  gtttgtaagc tcttgggtaa tggagtctat tccaacacct tccaacaggg
       e  f  v  s  s  w  v  m  e  s  i  p  t  p  s  n  r

 251  tgaaaacgca gcagaaatct aacctagagc tgctccgcat ctccctgctg
       v  k  t  q  q  k  s  n  l  e  l  l  r  i  s  l  l

 301  ctcatccagt catggctgga gcccgtgcag ctcctcagga gcgtcttcgc
        l  i  q  s  w  l  e  p  v  q  l  l  r  s  v  f

 351  caacagcctg gtgtatggcg cctcggacag caacgtctat cgccacctga
      a  n  s  l  v  y  g  a  s  d  s  n  v  y  r  h  l

 401  aggacctaga ggaaggcatc caaacgctga tgtggaggct ggaagatggc
       k  d  l  e  e  g  i  q  t  l  m  w  r  l  e  d  g

 451  agcccccgga ctgggcagat cttcaatcag tcctacagca agtttgacac
        s  p  r  t  g  q  i  f  n  q  s  y  s  k  f  d

 501  aaaatcgcac aacgatgacg cactgctcaa gaactacggg ctgctctact
      t  k  s  h  n  d  d  a  l  l  k  n  y  g  l  l  y

 551  gcttcaggaa ggacatggac aaggtcgaga cattcctgcg catcgtgcag
       c  f  r  k  d  m  d  k  v  e  t  f  l  r  i  v  q

 601  tgccgctctg tggagggcag ctgtggcttc caccatcacc atcaccattg
        c  r  s  v  e  g  s  c  g  f  h  h  h  h  h  h

 651  aaacccagct ttcttgtaca aagtggt
```

bold = signal sequence

Figure 9: Map of pENTR- INSP105-6HIS (plasmid 14855)

Molecule: pENTR-INSP105-6HIS, 3171 bps DNA Circular
File Name: 14855.cm5

Description: Ligation of B1b2-orf.seq* into pDONR221*

Type	Start	End	Name	Description
REGION	295	268 C	rrnB T2	transcription termination sequence
REGION	470	427 C	rrnB T1	transcription termination sequence
REGION	537	552	M13F	forward primer
REGION	570	651	attL1	
GENE	677	1291	INSP105-6HIS	
REGION	1306	1394	attL2	
REGION	1452	1436 C	M13 R	reverse primer
GENE	1565	2374	Kan r	
GENE	2495	3168	pUC ori	

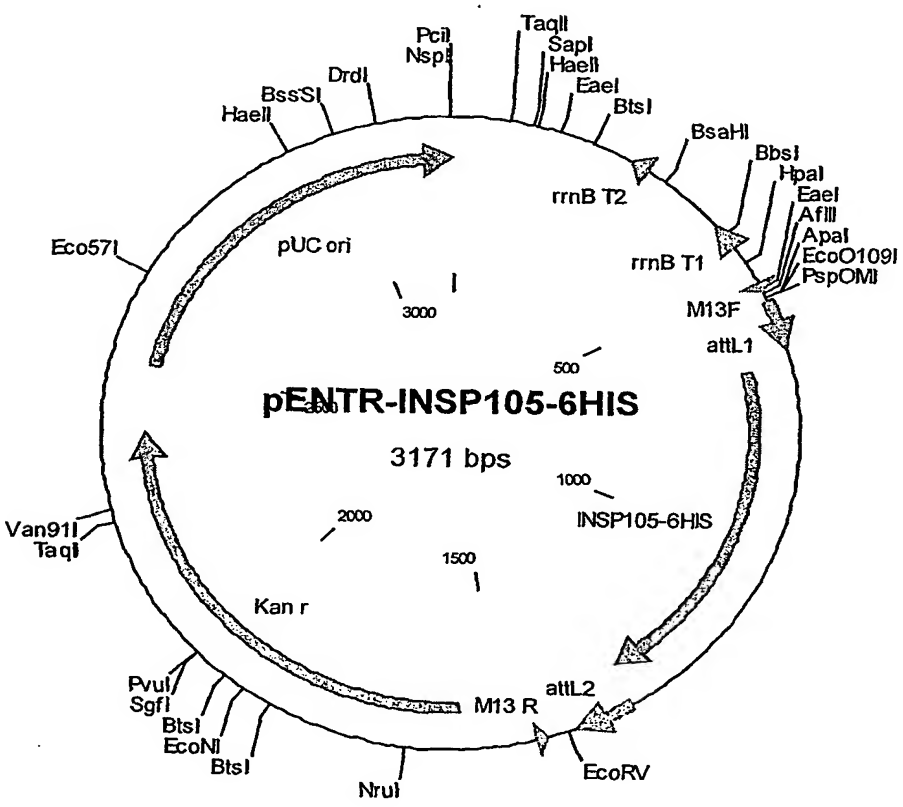


Figure 10: Map of pEAK12d- INSP105-6HIS (plasmid 14856)

Molecule: pEAK12d-INSP105-6HIS-V1, 7546 bps DNA Circular
File Name: 14856.cm5

Description: pEAK12 DES with two recombination sites attR1 and attR2 between which the cDNA is inserted

Type	Start	End	Name	Description
REGION	2	595	pmb-ori	
GENE	596	1519	Amp	
REGION	1690	2795	EF-1alpha	
REGION	2703	2722	peak12-F	forward primer
REGION	2855	2874	attB1	
GENE	2888	3484	INSP105-6HIS	
REGION	3492	3513	attB2	
REGION	3520	3948	'A	poly A/splice
REGION	3634	3615 C	peak12-R	reverse primer
GENE	4567	3949 C	PUR	PUROMYCIN
REGION	4791	4568 C	tK	tK promoter
REGION	5286	4792 C	Ori P	
GENE	7338	5286 C	EBNA-1	
REGION	7339	7538	sv40